JOURNAL OF BACTERIOLOGY, Apr. 1981, p. 18-23
0021-9193/81/040018-06$02.00/0

Vol. 146, No. 1

Effect of *gyrB*-Mediated Changes in Chromosome Structure on Killing of *Escherichia coli* by Ultraviolet Light: Experiments with Strains Differing in Deoxyribonucleic Acid Repair Capacity

ATTE VON WRIGHT AND BRYN A. BRIDGES*

MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton, Sussex BN1 9QG, England

Received 21 November 1980/Accepted 29 January 1981

Mutations at the *gyrB* locus were found to decrease the degree of supercoiling of the *Escherichia coli* chromosome. The effect of a *gyrB* mutation on the repair of ultraviolet-induced deoxyribonucleic acid damage was studied by following the killing of strains of *E. coli* K-12 proficient and deficient in deoxyribonucleic acid repair. The effectiveness of both excision and postreplication types of deoxyribonucleic acid repair was found to be altered by this mutation, the former being apparently enhanced and the latter impaired.

The *Escherichia coli* enzyme DNA gyrase, one of the DNA topoisomerases, is responsible for maintaining negative superhelicity in covalently closed circular DNA (5). It consists of at least two subunits, A and B, that have been identified as the *cou* and *nalA* (*gyrA*) gene products, respectively (1, 10).

The antibiotic novobiocin is an inhibitor of DNA gyrase and reacts specifically with the *cou* gene product, the B subunit of the enzyme. This has made it possible to isolate *E. coli* mutants (now designated *gyrB*) that are resistant to novobiocin or related antibiotics and contain an altered DNA gyrase B subunit (4, 11).

The *gyrB* mutants have been reported to be defective in DNA transcription, initiation of DNA synthesis, and cell division. This phenotype has been assumed to result from reduced superhelicity of the bacterial chromosome in these mutants (4, 11). To obtain information on the effects of the three-dimensional structure of DNA in the repair processes, we have studied two novobiocin-resistant *gyrB* point mutants, one temperature sensitive (11) and the other not (4). The effect of these two mutations on the superhelicity of the bacterial chromosome was checked by comparing the sedimentation properties of isolated nucleoids in the presence of ethidium bromide with those obtained from nearly isogenic *gyrB*$^+$ strains and, in the case of the thermosensitive mutant, from cells grown at permissive temperature. The effect on DNA repair was studied by comparing the killing by UV light of *gyrB*$^+$ and *gyrB* non-temperature-sensitive strains with different repair capacities.

We report that *gyrB* mutations do, indeed, reduce the superhelicity of the *E. coli* chromosome, and this reduction of superhelicity is accompanied by apparent enhancement of the excision repair process and by a reduction in the efficiency of postreplication repair.

MATERIALS AND METHODS

Strains and culture conditions. The strains used in this work are listed in Table 1. For the experiments the strains were routinely grown in Davis-Mingioli minimal medium (2) supplemented with 0.4% glucose, 0.4% Casamino Acids (Difco Laboratories), and 0.1 μg of thiamine per ml. This medium was also used as the solid plating medium in survival experiments.

Transduction of the *gyrB* mutation. Kanamycin-resistant P1 phage, temperature sensitive for lysogeny, was kindly donated by Frank Cannon, ARC Nitrogen Fixation Unit, University of Sussex. P1 lysogens of the *gyrB* strain LE701 were obtained by plating the bacteria together with the phage on plates containing 20 μg of kanamycin per ml and picking up the resistant colonies after overnight incubation at 32°C. The medium was modified L-agar (16) into which $CaCl_2$ was added to a final concentration of 5 mM.

The transducing lysate was prepared by inducing the lytic cycle in exponentially growing L-broth cultures of lysogens by adding $MgCl_2$ and $CaCl_2$ to final concentrations of 5 mM each and incubating for 15 min at 42°C. The cultures were subsequently transferred to 37°C for a further 2 h. After clearing, the lysates were treated with chloroform, centrifuged, and stored in the refrigerator.

The transduction was performed by plating the transducing lysate together with the recipients on L-agar plates containing, in addition to kanamycin and $CaCl_2$, 500 μg of novobiocin per ml. The novobiocin-resistant colonies were picked after overnight incubation at 32°C. They were cured of prophage by plating

TABLE 1. *Bacterial strains used*

Strain	Repair genotype	Other markers	Reference
LE234	Wild	*ilv arg met rps tra thi xyl*	4
LE701	Wild	As LE234 but *gyrB*	4
LE316[a]	Wild	As LE234 but *gyrB*(Ts)	11
AB1157	Wild	*thr leu arg pro thi lac str his xyl gal tsx*	7
AB1886	*uvrA*	As in AB1157	8
CM1113	Wild	As AB1157 but *gyrB*	This laboratory
CM1114	*uvrA*	As AB1886 but *gyrB*	This laboratory
CM1115	*recA56*	*recA* derivative of AB1157	This laboratory
CM1116	*recA56*	*recA* derivative of CM1113	This laboratory
CM1117	*uvrA recA56*	*recA* derivative of AB1886	This laboratory
CM1118	*uvrA recA56*	*recA* derivative of CM1114	This laboratory

[a] The *gyrB*(Ts) mutation is lethal to the cell with prolonged incubation at 42°C.

exponential-phase cultures on L-plates and incubating at 42°C overnight.

Construction of *recA* strains. The *recA* mutation was introduced into the test strains by first making them auxotrophic for thymine, using trimethoprim selection, and then mating them with the *recA56* Hfr strain JC5088 (3). The selection was for thymine independence, and streptomycin (0.3 mg/ml) was added to the selection medium to eliminate the donor. The recombinants were tested for UV sensitivity, and the sensitive clones were streaked on nutrient agar plates and used in further experiments.

Lysis procedures. The strains proved to be extremely variable in their responses to the lysis procedures. The method of Hecht et al. (6) produced satisfactory results with strain AB1157 and its *gyrB* derivative CM1113. [^{3}H]thymidine-labeled cells from 10-ml exponential-phase cultures were harvested and suspended in 0.4 ml of solution A (0.01 M Tris, 1.0 M NaCl, pH 8.1). The suspension was transferred into an ice bath, and 0.1 ml of solution B (10 mg of lysozyme per ml in 0.12 M Tris–0.05 M EDTA, pH 8.1) was added. After 2 min of incubation, 0.5 ml of solution C (1.0% Brij 58, 0.4% deoxycholate, 1.0% Sarkosyl NL-97, 1.0 M NaCl, 10 mM EDTA) was added, and the mixture was transferred to a 24°C water bath. The suspension was incubated for 3 min, during which time it cleared. When this method was applied to strains LE234 and LE701, however, a clear, nonviscous, but extremely slowly sedimenting lysate was formed. Reducing the concentration of Sarkosyl in the detergent solution to 0.25% (14) was found to be necessary with these strains to obtain good results. Neither modification of the Hecht procedure was successful with strain LE316, and the procedure of Ullmer et al. (15) had to be used. The lysozyme treatment was for 5 min at 25°C, followed by 3-min detergent treatment at 0°C. The concentration of Sarkosyl in solution C was 0.25% if the cells had grown at permissive temperature, but 1.0% was found to be necessary if they had been incubated at 42°C before lysis.

Sucrose gradient centrifugation. Portions of 0.25 ml of the lysates were layered on 4.2-ml 5 to 30% (wt/vol) sucrose gradients (in 0.01 M Tris, 1.0 M NaCl, 1.0 mM EDTA). The gradients were centrifuged at 17,000 rpm and 4°C for 20 min, using a Spinco SW50.1 rotor. To estimate the sedimentation coefficients, the gradients were calibrated by centrifuging ^{14}C-labeled T4 phage (1,025S) for various times.

After centrifugation the gradients were collected on strips of filter paper (Whatman grade 17), washed once in 5% trichloroacetic acid and twice in ethanol, dried, and counted, using a toluene-based scintillation liquid.

Estimation of DNA superhelicity. The degree of superhelicity of the isolated nucleotides was determined with ethidium bromide-containing gradients and by following the sedimentation of the nucleoids as a function of ethidium bromide concentration (14). According to theory, ethidium bromide causes the relaxation of negatively supercoiled DNA due to its intercalating action. Consequently, the sedimentation coefficient of the DNA decreases with increasing concentration of ethidium bromide.

This continues until the chromosome becomes completely relaxed, after which, because of the continued intercalation of ethidium bromide, it starts to become positively supercoiled. This results in an increase in sedimentation coefficient. Thus, the concentration of ethidium bromide at which the sedimentation coefficient of the DNA starts to increase after the initial relaxation is an indication of the degree of the original negative superhelicity of the DNA in question.

Comparison of the UV sensitivities of *gyrB*$^+$ and *gyrB* strains. The strains were grown into the exponential growth phase, harvested, and suspended in phosphate buffer. The suspensions were irradiated with 254-nm UV light obtained from a Phillips FUV 6W lamp calibrated with a Latarjet meter. Serial dilutions of the irradiated suspensions were plated, and the plates were incubated overnight at 37°C or, if the temperature-sensitive strain LE316 was being used, at 32°C. The colonies were then scored, and the viable counts of bacteria were determined.

RESULTS

Effect of *gyrB* mutation on the superhelicity of the bacterial chromosome. The results of the ethidium bromide titrations of the degree of DNA superhelicity in isolated nucleoids are expressed in Fig. 1. It can be seen that in all three *gyrB* strains tested the concentration of ethidium bromide needed to reverse the initial lowering of the sedimentation constant caused by this agent was lower than in corresponding isogenic wild-type strains. This was true despite the differences in sedimentation

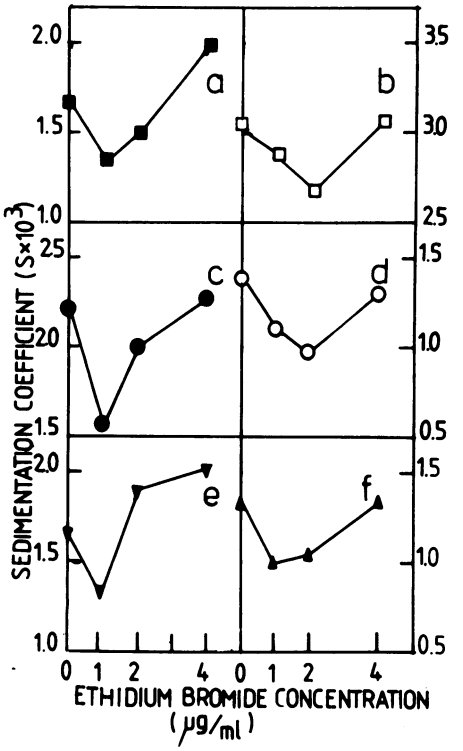

FIG. 1. *Effect of ethidium bromide on the sedimentation of nucleoids isolated from gyrB strains and their wild-type parents. The gyrB strains in (a), (c), and (e) are CM1113 (■), LE701 (●), and LE316(Ts) (▼) incubated at 42°C for 1 h before the experiment. The corresponding wild-type controls in (b), (d), and (f) are AB1157 (□), LE234 (○), and LE316(Ts) (▲) incubated at 32°C. Points represent the means of three experiments. Approximately 60,000 cpm were layered on each gradient.*

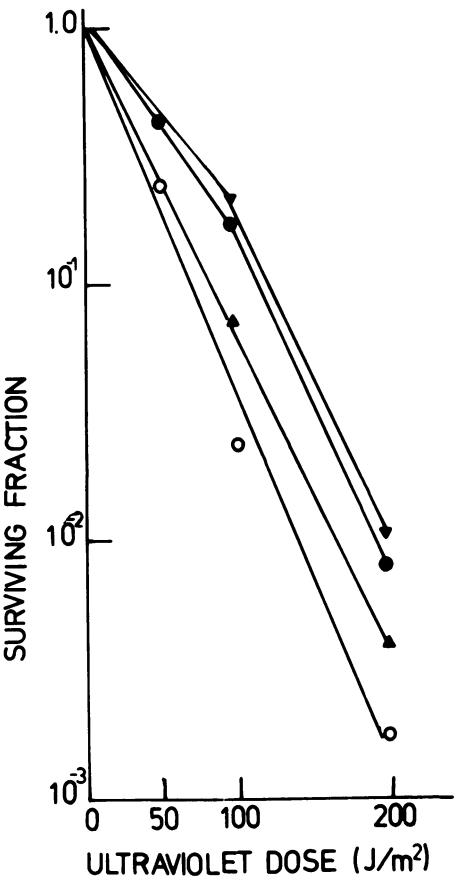

FIG. 2. *UV killing of strain LE234 (○) and its gyrB derivatives LE701 (●) and LE316. The thermosensitive gyrB mutant LE316 was either grown at 32°C throughout the experiment (▲) or incubated at 42°C for 1 h before irradiation (▼). Points represent the means of three experiments.*

constants caused by different lysis methods and indicates that the superhelicity of the bacterial chromosome is reduced in strains carrying mutations at the *gyrB* locus. In the temperature-sensitive strain LE316 there was a suggestion of reduced superhelicity (compared with LE234) even at permissive temperature.

UV sensitivities of strains LE701 and LE316 compared with parental strain LE234. The *gyrB* strain LE701 was more resistant to UV than was its parent strain LE234 (Fig. 2). The same was true with the thermosensitive *gyrB* mutant LE316 if the strain was incubated at 42°C, but the effect was marginal if the strain had been grown throughout at 32°C. This effect at 32°C may well be real, however, given the apparent reduction in superhelicity even at permissive temperature. Orr (personal communication) has observed altered nuclear morphology, cell division pattern, and DNA replication in LE316 at permissive temperature, and no gyrase activity could be detected in cell-free extracts at permissive temperature.

Effect of the *gyrB* mutation on UV sensitivity in the AB1157 background. In contrast to strains LE701 and LE316, the *gyrB* derivative of AB1157 (CM1113) was only marginally more resistant to UV than was AB1157 and the *gyrB* derivative of the *uvrA* strain AB1886 (CM1114) was about twice as sensitive to UV as was the original strain. The repair-proficient strain AB1157 was, moreover, found to be more resistant to UV than the other parental strain LE234 (Fig. 3 and 4).

The presence of a *recA* mutation in the excision-proficient test strains revealed a marked difference in the effect of the *gyrB* allele, strain CM1116 (*recA gyrB*) being much more resistant than CM1115 (*recA gyrB*$^+$) (Fig. 5). No such

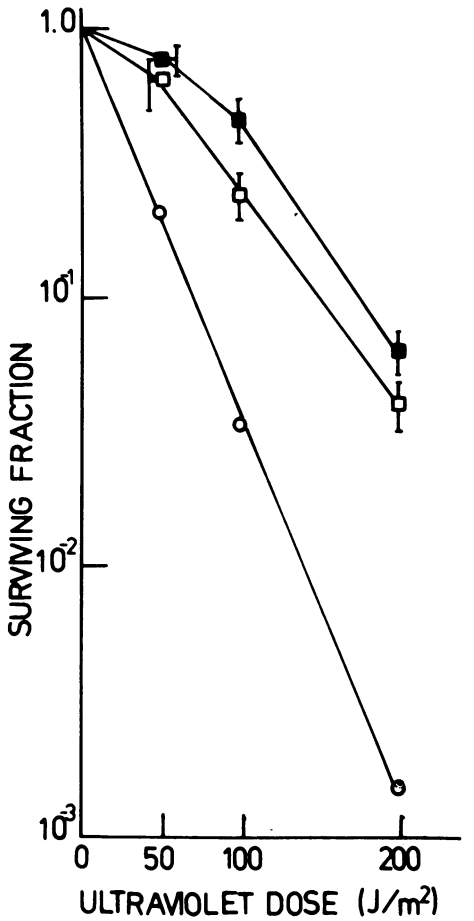

FIG. 3. *UV killing of strain AB1157* (□), *its gyrB derivative CM1113* (■), *and strain LE234* (○). *Bars indicate the standard error of three experiments.*

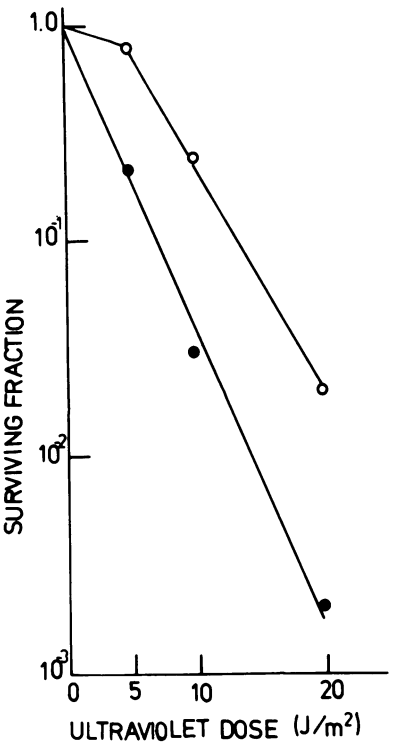

FIG. 4. *UV killing of uvrA strain AB1886* (○) *and its gyrB derivative CM1114* (●). *Points represent the means of three experiments.*

difference could be detected between the excision-deficient CM1117 (*uvrA recA gyrB*$^+$) and CM1118 (*uvrA recA gyrB*) strains (Fig. 6).

DISCUSSION

Despite the differences in sedimentation rates, all of the *gyrB* strains had their turning points in ethidium bromide titration experiments at lower ethidium bromide concentrations than the wild-type strains, indicating a lesser degree of superhelicity in their chromosomes.

The present data do not give any indication as to whether superhelicity is generally lower in *gyrB* strains throughout the chromosome or whether some regions have normal superhelicity and others do not. This would be possible if the chromosome were organized into independent domains as far as superhelicity is concerned (see 17).

It became apparent in UV irradiation experiments that a *gyrB* mutation, presumably through its effects on DNA superhelicity, enhanced survival after UV irradiation in bacteria possessing only excision repair and decreased it in bacteria possessing only postreplication repair. In bacteria with both pathways active, the result depended upon the genetic background of the cell. To understand these differences it is necessary to consider first the results with repair-deficient strains. Strains carrying both the *recA* and *uvrA* mutations are completely deficient in dark repair of UV damage and are unable to tolerate more than one (or at the most two) pyrimidine dimers in the DNA (9). That the *gyrB* mutation has no effect when introduced into such a strain establishes that the degree of superhelicity of the chromosome does not significantly alter the yield of UV-induced pyrimidine dimers in DNA. We may then deduce that the effects on survival observed in strains possessing functional DNA repair pathways are attributable to the influence of *gyrB* on these pathways.

In *uvrA* bacteria, only postreplicational pathways are operational, and the considerably greater lethality observed when the *gyrB* mu-

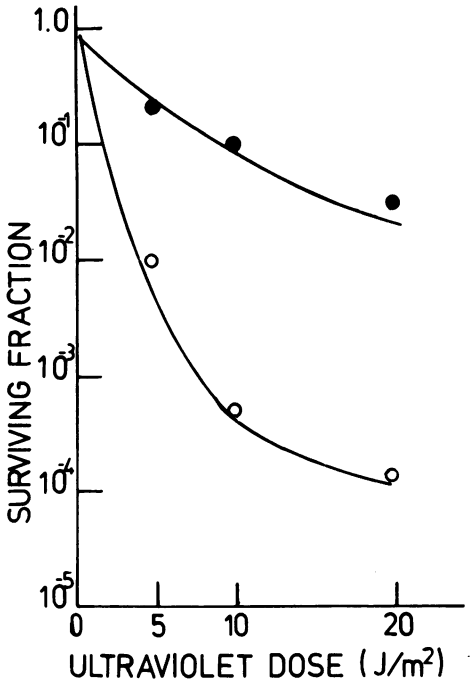

FIG. 5. *UV killing of recA strain CM1115* (○) *and the near-isogenic recA gyrB strain CM1116* (●). *Points represent the means of three expeirments.*

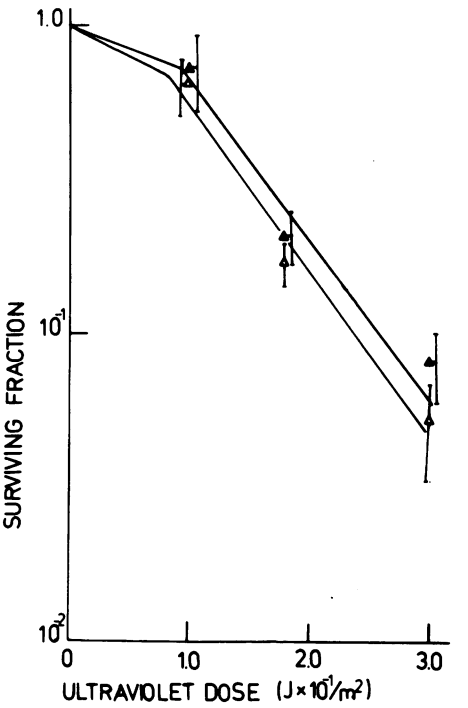

FIG. 6. *UV killing of recA uvrA strain CM1117* (△) *and the near-isogenic recA uvrA gyrB strain CM1118* (▲). *Bars indicate the standard errors of three experiments.*

tation was present shows that this mutation impairs the functioning of postreplication repair. The major pathway of postreplication repair appears to involve recombinational exchanges between daughter chromosomes at the sites of daughter strand gaps (12). Such exchanges are likely to be initiated by the invasion of the undamaged daughter strand by a single-stranded end from the damaged strand (see 13). Insofar as such an invasion is likely to be facilitated by negative superhelicity in the undamaged daughter, it is not unreasonable to interpret the consequences of the *gyrB* mutation as being to reduce superhelicity in the undamaged daughter chromosome and thus to inhibit the initiation of daughter strand exchanges. Consistent with this is the observation that the *gyrB* derivatives of AB1157 and AB1886 (CM1113 and CM1114, respectively) are recombination deficient, producing only about 20% of the number of His^+ recombinants in a cross with the Hfr strain JC5088, compared with the gyr^+ strains (unpublished data).

In *recA* bacteria there is no postreplication repair but prereplicational excision processes continue, albeit probably to a reduced extent. In such bacteria the presence of a *gyrB* mutation results in enhanced survival, suggesting that excision repair is promoted by *gyrB*. This suggests that the negative superhelicity normally found in the *E. coli* chromosome to some extent restricts excision repair.

It is not, however, necessary to envisage an influence of superhelicity on the efficiency of the incision step to explain the results. In *recA* bacteria, for example, it could be that a certain proportion of incisions normally leads to DNA degradation and death and that the reduction in superhelicity leads to a lowering of the probability that this will occur. A more trivial explanation would be that DNA replication is slower in *gyrB* bacteria, which allows more time for prereplicative excision repair.

It must also be remembered that because of the many ways in which the *recA* mutation affects cellular response to DNA damage, it cannot be assumed that any effect of *gyrB* in a *recA* background will also occur in a rec^+ background. If this assumption were made, however, since both excision and postreplication repair pathways operate in Uvr^+ Rec^+ strains, the overall effect of the *gyrB* mutation is likely to depend upon the relative contribution of the two processes to survival. Any tendency to increased survival due to enhanced excision repair would tend to be opposed by a decrease due to less effective postreplication repair, the balance between these being difficult to predict. Our results suggest that the *gyrB* impairment of postrepli-

cation repair may be generally less important than the promotion of excision repair in LE234 and AB1157.

LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. J. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116-167.
2. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
3. **Donch, J., M. H. L. Green, and J. Greenberg.** 1968. Interaction of the *exr* and *lon* genes in *Escherichia coli*. J. Bacteriol. **96**:1704-1710.
4. **Fairweather, N. F., E. Orr, and J. B. Holland.** 1980. Inhibition of deoxyribonucleic acid gyrase: effects on nucleic acid synthesis and cell division in *Escherichia coli* K-12. J. Bacteriol. **142**:153-161.
5. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3872-3876.
6. **Hecht, R. M., D. Simpson, and D. Pettijohn.** 1977. Sedimentation properties of the bacterial chromosome as an isolated nucleoid and as unfolded DNA fiber. Chromosomal DNA folding measured by rotor speed effects. J. Mol. Biol. **111**:257-277.
7. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119-1136.
8. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**:1137-1150.
9. **Howard-Flanders, P., L. Theriot, and J. B. Stedeford.** 1969. Some properties of excision-defective recombination deficient mutants of *Escherichia coli* K-12. J. Bacteriol. **97**:1134-1141.
10. **Hunsen, F. G., and K. von Meyenburg.** 1979. Characterization of the *dnaA*, *gyrB* and other genes in the *dnaA* region of the *Escherichia coli* chromosome on specialized transducing phages λ*tna*. Mol. Gen. Genet. **175**:135-144.
11. **Orr, E., N. F. Fairweather, J. B. Holland, and R. H. Pritchard.** 1979. Isolation and characterisation of a strain carrying a conditional lethal mutation in the *cou* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **177**: 103-112.
12. **Rupp, W. D., C. E. Wilde, D. L. Reno, and P. Howard-Flanders.** 1971. Exchanges between DNA strands in UV-irradiated *Escherichia coli*. J. Mol. Biol. **61**:25-44.
13. **Shibata, T., C. DasGupta, R. P. Cunningham, and C. M. Radding.** 1979. Purified *Escherichia coli recA* protein catalyzes homologous pairing of superhelical DNA and single-stranded fragments. Proc. Natl. Acad. Sci. U.S.A. **76**:1638-1642.
14. **Snyder, M., and D. Drlica.** 1979. DNA gyrase on the bacterial chromosome: DNA cleavage induced by oxolonic acid. J. Mol. Biol. **131**:287-302.
15. **Ullmer, K. M., R. F. Gomez, and A. J. Sinskey.** 1979. Ionizing radiation damage to the folded chromosome of *Escherichia coli* K-12: sedimentation properties of irradiated nucleoids and chromosomal deoxyribonucleic acid. J. Bacteriol. **138**:475-485.
16. **von Wright, A., and B. A. Bridges.** 1980. Effect of *tif* expression, irradiation of recipient and presence of plasmid pKM101 on recovery of a marker from a donor exposed to ultraviolet light prior to conjugation. J. Gen. Microbiol. **119**:327-331.
17. **Worcel, A., and E. Burgi.** 1972. On the structure of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **71**:127-147.